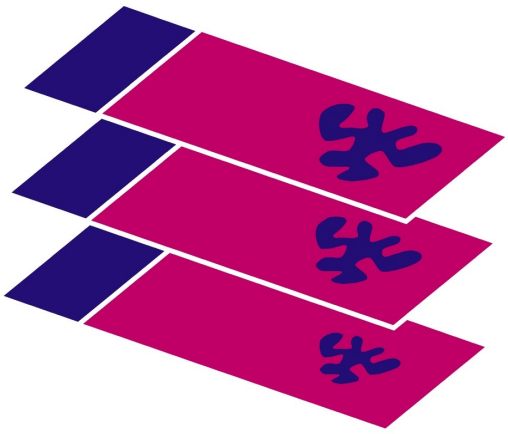

3DHISTECH

IH-Lab Module

1.11

User's Guide

June 30, 2008

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Font Types and Symbols

Example	Abbreviation or term that is explained in section (page).
Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task.
Important!	Contains an important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	Contains a suggestion about using the application in some other way or to some interesting purpose.

Terms and Abbreviations

Annotation	Area of interest on the digitized slide.
Consolidator	An additional user in a multi-user IH-Lab project who can see the scores given by all the other users and who can consolidate them.
Cumulative CSV export	Summary line appended to an existing CSV file in which you compile longitudinal or otherwise cumulative data.
Evaluator	A user who checks each field in each grid on each slide and assigns it a score. In a multi-user IH-Lab project, evaluators do not see each other's scores.
Field	A cell of the grid that is fitted onto an area of interest on the virtual slide. If you put a grid on slides from the same specimen, you can analyze the marker expression of the same fragment immunostained for different antigens. IH-Lab projects allow you to correlate the different antigens and perform measurements (for example with a separately available MIRAX Viewer module, HistoQuant).
Grid	A set of annotations used in IH-Lab. They are independent of other annotations, for example HistoQuant annotations that might be smaller than a grid field. Unlike plain MIRAX annotations or HistoQuant annotations, grids are not saved with the slides by default. Grid information is stored with the IH-Lab project in which you created or modified them but you can also save them onto the slides.
IH-Lab Gallery	An IH-Lab gallery contains all fields from all grids from all slides in the project For example, if you used a 2x3 grid and marked an area with the grid on 3 slides, the gallery for this stain on this specimen contains 2x3x3=18 images. In gallery mode, you can magnify small parts (the grid fields) to score them and to export them for reporting or in an image gallery.
IH-Lab project	An IH-Lab project is a set of slides that you want to evaluate with each other. In multi-user IH-Lab projects only the Consolidator can modify the project (add or remove slides, add or remove stains).
IHC	Immunohistochemistry
Marker	A stain, for example hormone (Estrogen-Receptor, Progesteron-Receptor, Her-2, EGFR, etc.).
Stain	Classic pathological stains, for example HE.

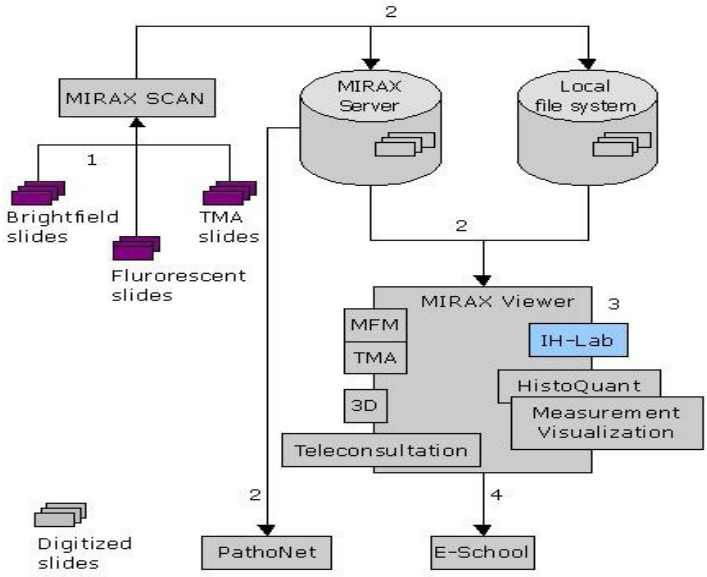
1 About the IH-Lab Module 1.11

IH-Lab is an optional module for MIRAX Viewer. It allows you to perform (semi)quantitative histopathological examinations of IHC-stained slides, digitized with MIRAX scanners.

- In the routine pathology, you can use IH-LAB to check specimens dyed with common IHC stains. Such an IH-Lab project typically contains sequential cuts from the same specimen and each slide is treated with a different stain.
- Research projects often use IH-Lab to check cuts from different specimens, for example from biopsies from the same patient, taken at different points in time. In such cases the slides would be dyed with the same marker.

1.1 IH-Lab Module in the MIRAX Product Family

For an overview of the workflow, see ***MIRAX Viewer - User's Guide***.



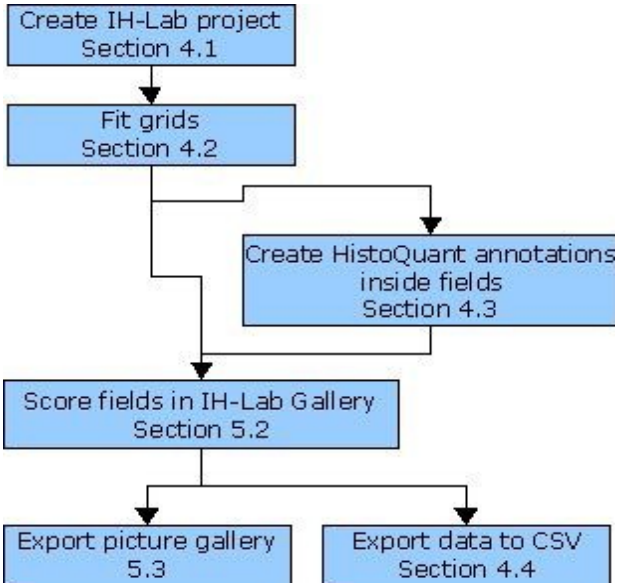
With the IH-Lab module, you can perform an immunohistochemical analysis on a slide set digitized with MIRAX scanners and stored on a local file system or on a MIRAX Slide Server.

1.2 Overview of the IH-Lab Module 1.11

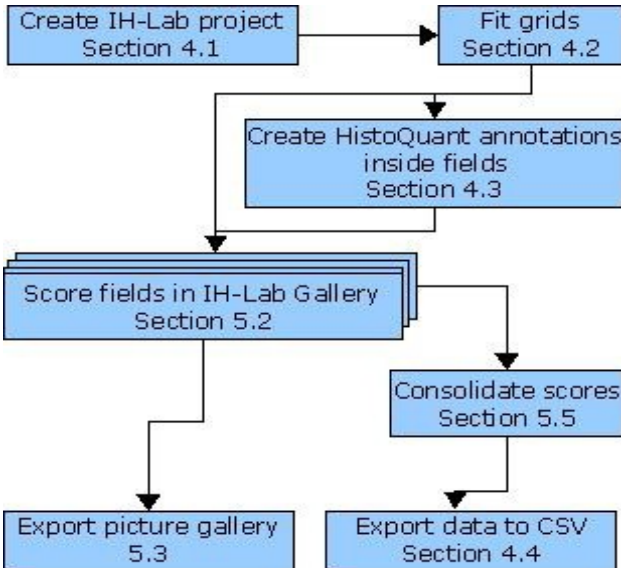
IH-Lab supports decision making in targeted therapy or in marker validation. It also makes your evaluations more reliable: with IH-Lab, you make subjective slide scoring more accurate and you make it measurable because you compare areas and scores on digitized MIRAX slides, which is a more reliable method than analyzing the actual slides empirically under traditional microscopes.

1.2.1 Workflow

The following figure summarizes your workflow:



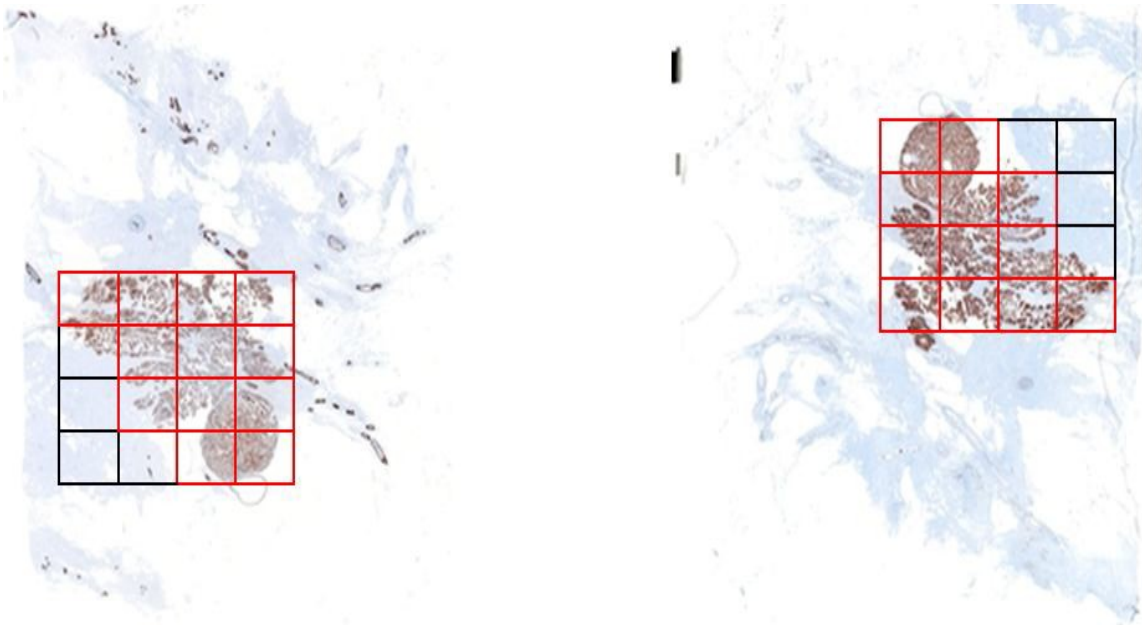
You can also use IH-Lab together with your colleagues. In this case, each team member scores the slides, and then one team member consolidates the scores.



For instructions on how to consolidate scores, see 8 Working with IH-Lab in Multi-User Mode (p. 29).

1.2.2 Grids

In routine pathology, an IH-Lab project contains slides from the same specimen. In such cases, grids are typically put on the same area on each slide, for example on a group of cancer cells.



Typically used grid setups:

n x m grid	You can put a grid on the area of interest and obtain data about smaller areas (grid fields). You can fit grids on the same area on several slides. IH-Lab helps you rotate and align the grid throughout several cuts from the same specimen. This allows you to obtain a report at object level (for example, about the nuclei found in the field) and also at the level of the field (for example, the number of positive cells compared to the number of negative cells). You can also knock out fields which are not interesting or not needed for the evaluation (for example, no tumor cells in it)
1 x 1 grid	For better sampling from the specimen if one n x m grid would be too large
Several grids	You can put several grids on the slides (scoring is done per annotation, that is, per grid field, IH-Lab calculates the final score per slide)
Grid-internal annotations	You can also create annotations that are smaller than the grid fields. You can use grid annotations and non-IH-Lab annotations on the same slide without any problems. If you want to use HistoQuant, you can run HistoQuant algorithms on either type of annotations.
Grids on several slides in the project	You can analyze several slides in a project and get a better overview of a specimen. Furthermore, you can export the data into one report.

1.2.3 Scoring Schemes

You can define a separate scoring scheme for each stain and several users can evaluate the slides. A Consolidator user reviews the scores and IH-Lab computes the final score for each annotation (each field in each grid on each slide).

1.2.4 Reusing Stain Definitions and Scoring Schemes

You can reuse stains and scoring schemes defined in other IH-Lab projects. The definitions are saved in a file (IHLabConfig.xml) and you can copy it to the machine of other MIRAX Viewer users.

1.2.5 Multi-User Mode and Score Consolidation

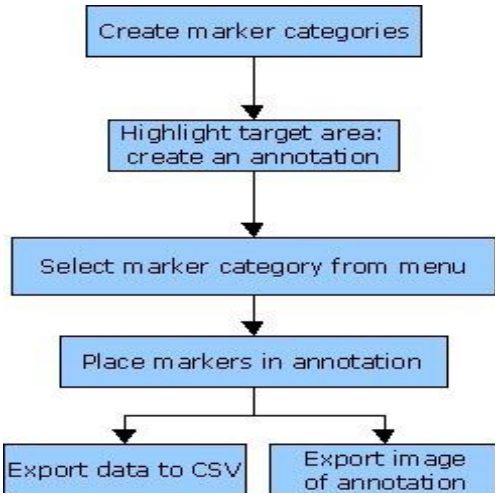
IH-Lab can handle scores from several evaluators and average their results. It also creates a Consolidator user for each multi-user report to facilitate score consolidation.

1.3 Marker Counter

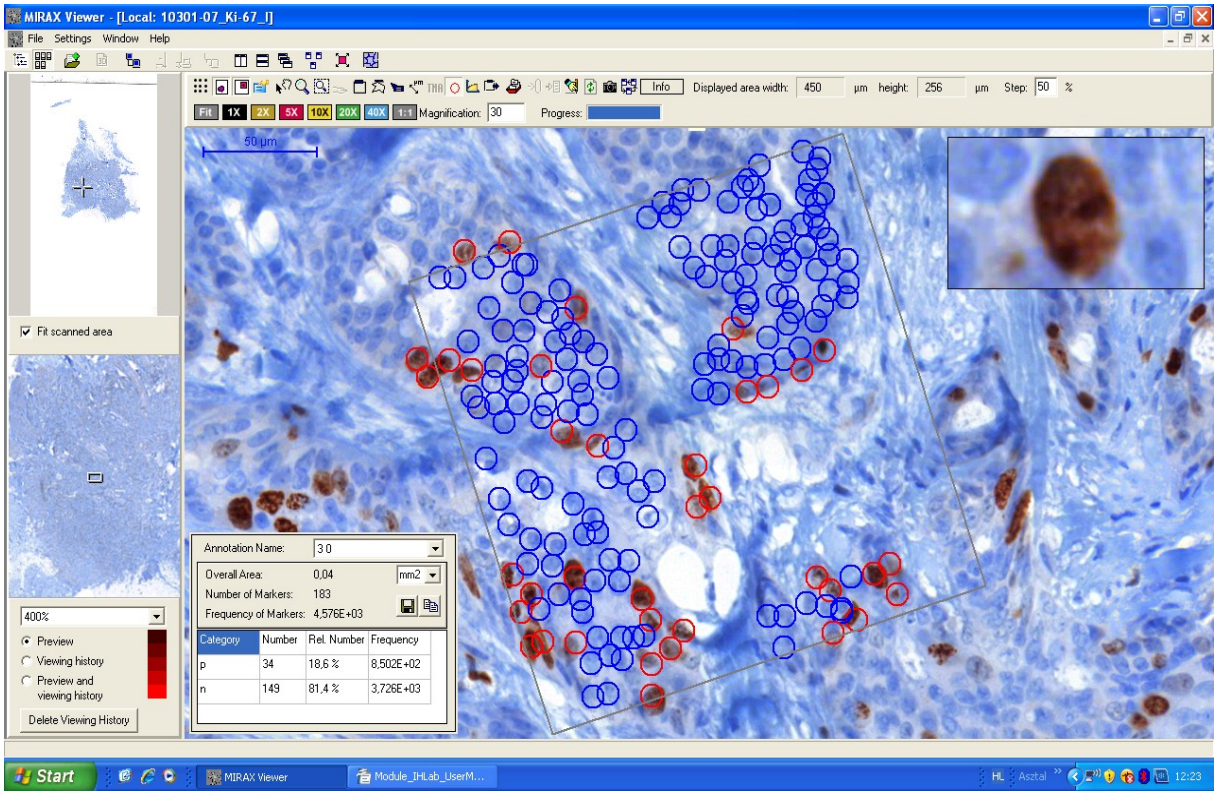
MIRAX Viewer includes Marker Counter to count cells and objects manually. You can move and reset Marker Counter on the screen and you can define the counter categories Marker Counter uses.

Marker Counter automatically generates the values and shows them in a pop-up window. You can use this tool for example to keep track of the following data in an annotation or per a standard area, for example 1 $\mu m^{2.}$. Marker Counter determines the number of marked objects, percentage of positive cells, and as derived information the individual or average intensity of marked objects within their category

All measured parameters can be stored in the digital slide or they can be exported to database.


Create marker categories
Highlight target area: create an annotation
Select marker category from menu
Place markers in annotation
Export data to CSV
Export image of annotation

And an example of using marker counter:



1.4 IH-Lab and HistoQuant / Measurement Visualization

You can also use IH-Lab with other MIRAX Viewer modules:

Warning!

Each MIRAX Viewer module is licensed separately.

- With HistoQuant, the module that allows you to define and run measurement algorithms on MIRAX annotations, you can perform the measurements on the entire slide set in an IH-Lab project.
- With Measurement Visualization, you can display the HistoQuant measurements as a scatterplot, a histogram, or as a gallery of pictures. You can also export your results into a CSV file or as an HTML picture gallery.

2 Installing the IH-Lab module for MIRAX Viewer

2.1 Licensing of MIRAX Products

Mirax software is purchased with a valid license file (Mirax.lic) which the software needs to exploit its all options. Without this file the Mirax Viewer offers only limited options as a free Mirax Viewer.

2.2 Prerequisites

IH-Lab is not a standalone application: you need MIRAX Viewer to run it. Therefore, it is installed together with MIRAX Viewer, bundled into one executable.

MIRAX Viewer version	1.11 Running on Microsoft Windows XP
Hardware	**minimum**: Pentium P4, 3GHz, 1GB RAM **recommended**: Pentium C2D (DualCore), 2 GHz, 2GB RAM
Disk space	MIRAX Viewer bundled with the IH-Lab module **minimum**: 88 MB

2.3 Installing the Application Bundle

1. Start the **setup.exe** file that you received on a DVD or downloaded in a compressed file.

2. Follow the instructions in the installation wizard.

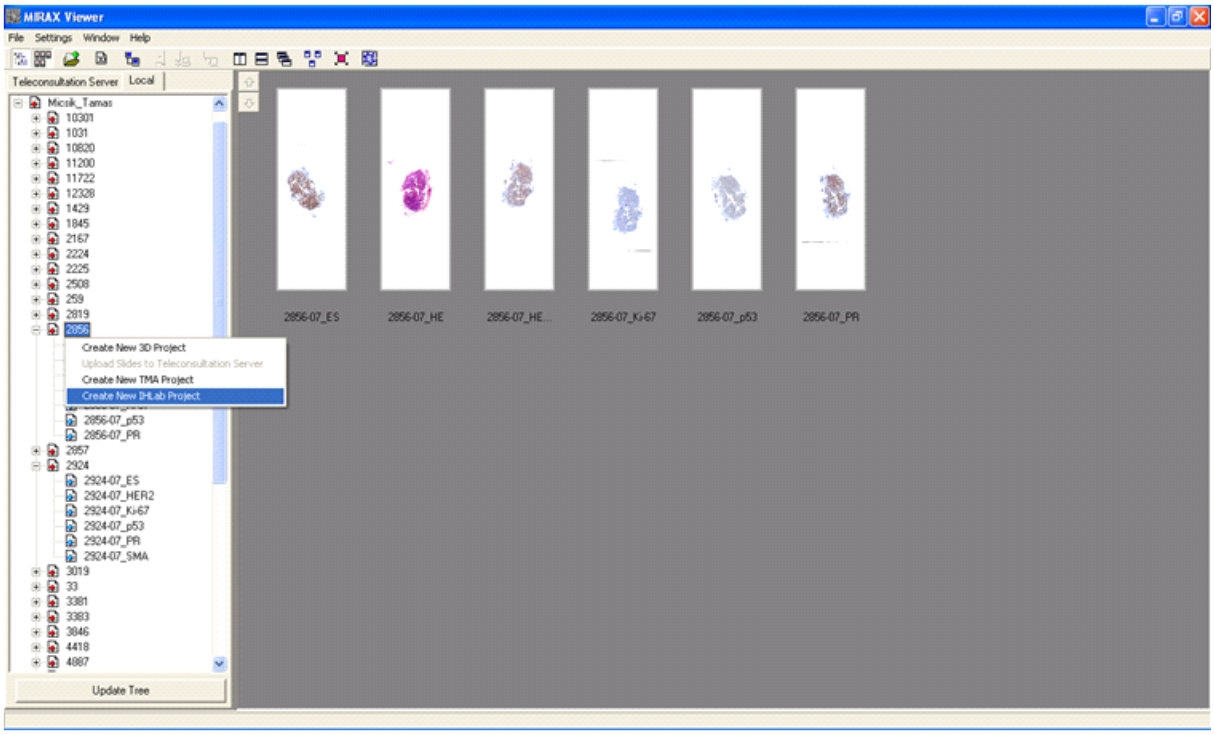
3 Creating an IH-Lab Project

Before You Start

Make sure that the slides that you want to add to the IH-Lab project are all in one folder at a location that you can access from MIRAX Viewer.

3.1 Create New IH-Lab Project

IH-Lab projects are handled and created within Mirax Viewer. In the Project Tree, right-click the folder in which you want to create the IH-Lab project, select **Create new IH Lab Project** from the menu and enter a name for the new project.



Now IH Lab Project Wizard is launched and helps You to go through all steps.

3.2 Give Name for the New IH-Lab Project

As name cannot be modified later be cautious to incorporate all needed information into the name of the new project.

The IH-Lab Project will be located within the folder of its slides, which can be visualized after pushing 'update Tree' button in the left side of the Mirax Viewer screen.

3.3 Define at Least One User on User Management Screen

Enter a user (name and ID) who will score the slides.

If you need a multi-user project, you can add further users later on when the project has been created and you first start the project.

3.4 Project Data

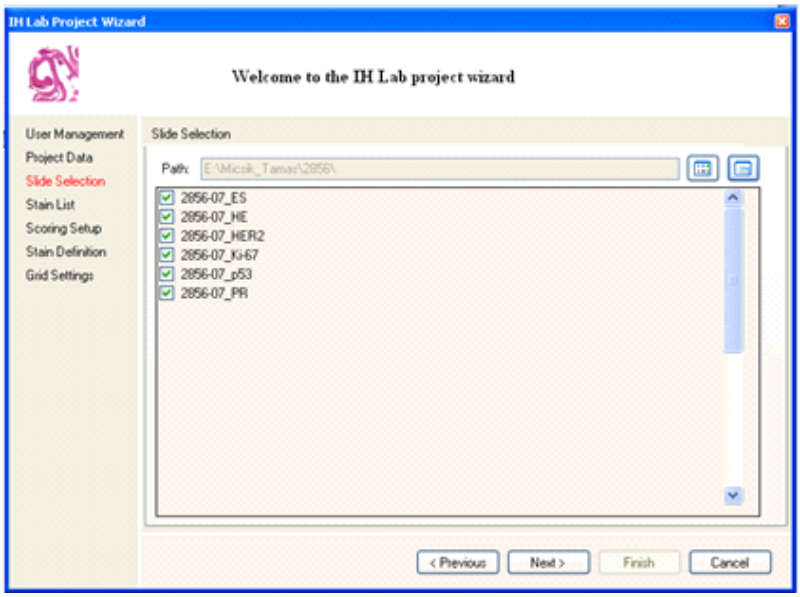
You may add parameters and values for the project, for example patient name(s) and the corresponding IDs or registry numbers, if it is needed. You may skip this option.

3.5 Slide Selection

You can see all slides in the actual folder. Select or clear checkboxes to create the slide set that you need for this IH-Lab project.

For multiple selection You can select several slides at the same time with Shift+Click and/ or Ctrl+Click. (This option is also valid for the further steps in the Wizard.)

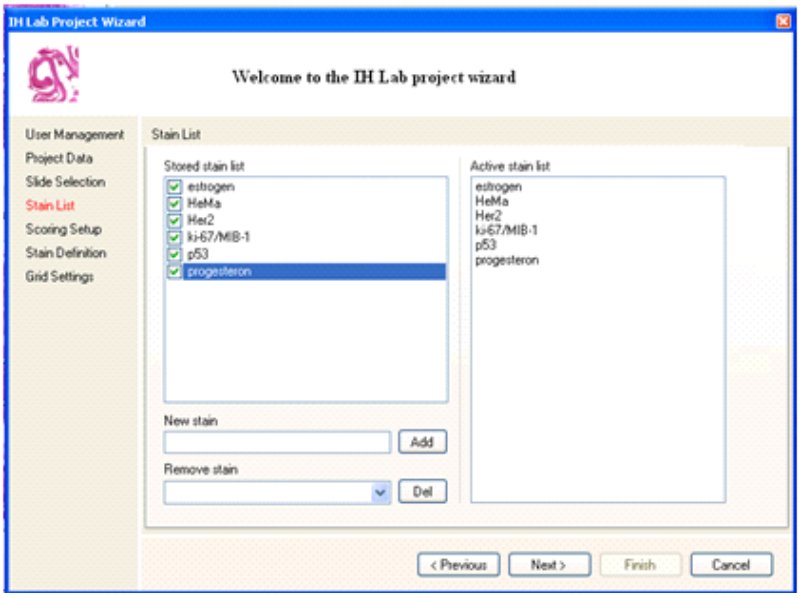
The slides that you leave out are not removed from the file system. You can also add them or new ones to the project later.



3.6 Stain List

Specify which markers are used in the actual project. Use checkboxes to add marker for the marker list.

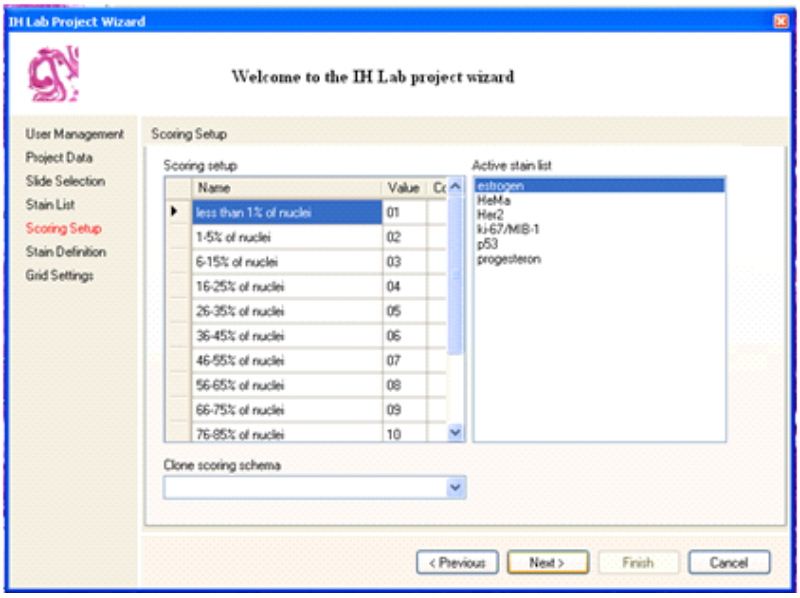
You can also add new stains here.



3.7 Scoring Setup

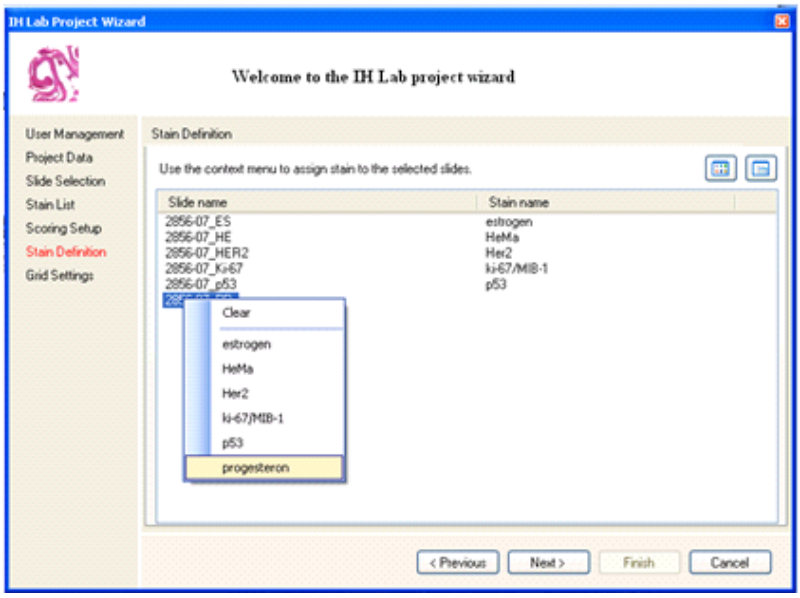
Create a scoring scheme for the stains. You can assign a different scheme (numeric value and color) to each stain. You can also speed up scheme generation by cloning or reusing existing schemes.

You can also use the available scoring schemes. If you modify them for a stain, the scoring scheme is saved with the changes for this stain but the scoring scheme is not changed for the other (“old”) stain.



3.8 Stain Definition

Mark for each slide which stain is applied to it. You can select several slides with Shift+Click and Ctrl+Click and apply the same stain marker to them in one step.



The wizard asks you whether you want to start fitting the grid onto the slides right away. You can do grid fitting later. To start grid fitting right away, select the checkbox in the upcoming window.

3.9 Grid Definition

Important!

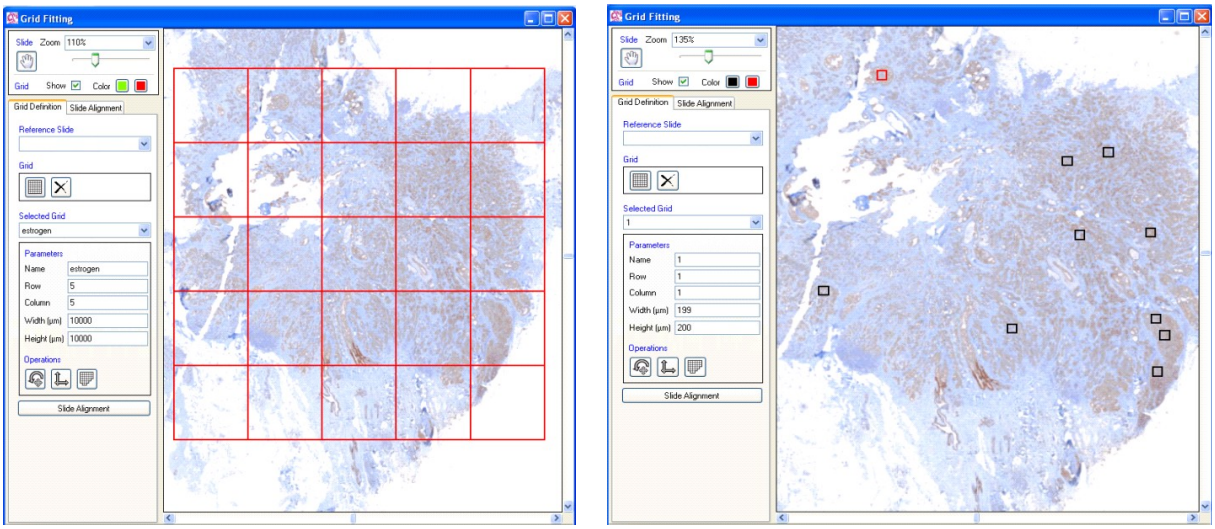
In multi-user projects only the Consolidator user can fit grids to the slides.

On each slide, mark the areas that you want to inspect, for example areas with suspected cancer, with one or more grids.

Specify the grid properties: name, number of rows and columns, the size of the grid in µm.

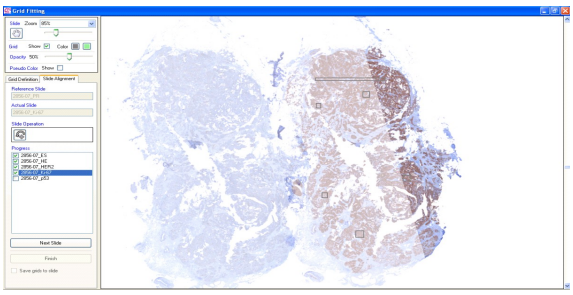
You can also adjust the position the grid on the slide, or rotate, enlarge/shrink it by dragging the grid borders.

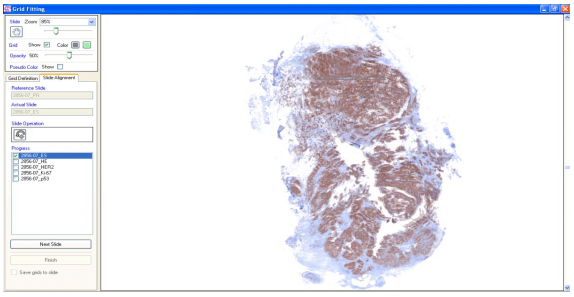
You can also put several grids on the slides: click the **New Grid** button.



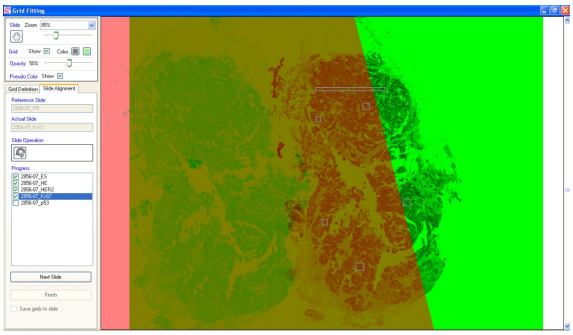
3.10 Grid fitting

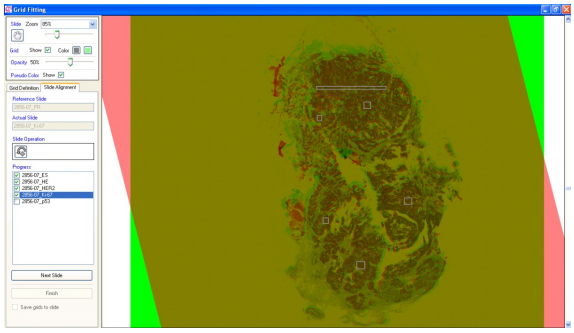
Go through all slides pairwise and align them onto each other: move (left mouse button) or rotate (right mouse button) the slides so that the grids exactly cover the same area on each of them.





Using Pseudo Color option helps You to better adjust slides.





Repeat these steps for each slide that you want to use in this IH-Lab project.

Save the grid to the slide, if You want to further analyze annotations with Marker Counter or Histoquant.

Click **Finish** to create the IH-Lab project.

3.11 Creating Annotations Inside an IH-Lab Grid

IH-Lab grids are annotations that you create when you create the IH-Lab project. You can nevertheless use further annotations on the slides. There might be already annotations on a slide or you might want to create HistoQuant annotations that are smaller than the grid field: if you have the optional HistoQuant module, you can perform measurements on the HistoQuant annotations.

1. Open the slide in MIRAX Viewer.
2. Create the annotation on the area of your interest:
    a. Select the annotation type (rectangle, free-hand or point) and cover the area with the annotation.
    b. Add a caption to the annotation and enter a description.

       If you are using the HistoQuant module: only captions are exported to the CSV output file. Annotation descriptions are for internal use in MIRAX Viewer and HistoQuant.
3. Repeat step 2 for each area on which you want to run the algorithm.

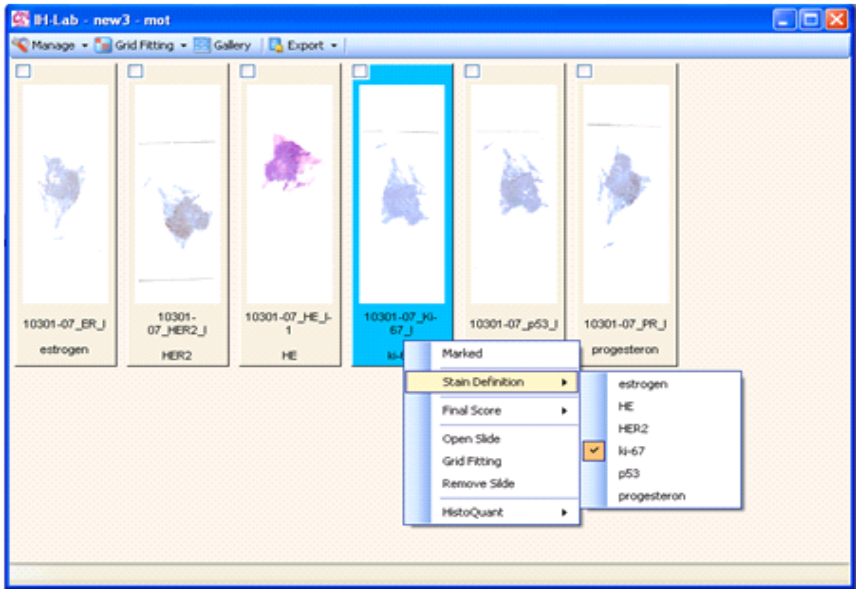
You will be able to run other algorithms on these annotations.

4 Handling an IH-Lab Project

4.1 Options in the IH-Lab Project

You can manage slides, stains; perform grid fitting, open gallery and export options from IH-Lab **Toolbar** or by directly choosing from the option by right clicking the mouse on the slide.

You can also mark slides, give a final score or open the slide or HistoQuant module.



4.2 Modifying an IH-Lab Project

Important!

If you modify an IH-Lab, the scores related to the slide/stain or grid are lost for all users who have already submitted scores to the project.

Most of the settings of an IH-Lab project can be modified.

In 'Single-user' mode the user can modify the project, but in 'Multi-user' mode only the consolidator user can modify IH-Lab projects:

- which slides are included in the project (they can add or delete slides)
- which stains are used in the project (they can add or delete stains)
- how many grids are used in the project (they can add or delete grids)
- grid layout (they can modify the size of a grid, they can change the number of rows or columns, they can knock out fields or put fields back into the grid)

4.3 Adding or Removing Slides

The way of adding or removing slides from the IH-Lab project:

1. Start the IH-Lab project.
2. Select **Manage > Add / Remove Slide(s)**.
3. Add or remove slides as needed:
   - Browse for slides to add.
   - Clear the checkbox of the slide(s) that you want to remove.
4. Click **OK** to update the IL-Lab project.

The way of removing a slide quickly:

1. Start the IH-Lab project.
2. Right-click the slide to be removed and then select **Remove Slide**.

4.4 Adding or Removing Stains

1. Start the IH-Lab project.
2. Select **Manage > Stain Manager**.
3. Add or remove stains as needed and then click **OK**.

4.5 Changing the Grid

Important!

In multi-user projects only the Consolidator user can fit or modify grids.

1. Start the IH-Lab project.
2. Start the Grid Fitting wizard:
   - Select **Grid Fitting** and then select the appropriate option (**For All Slides** or **For Selected Slides**).
   - Right-click a slide for which you want to change the grid and then select **Grid Fitting** from the shortcut menu.
3. Modify the grid as needed.
4. Save the changes: click **Finish** on the **Slide Alignment** tab.

5 Working with an IH-Lab Gallery

5.1 Enlarging the Preview Window

To make the preview window as large as possible, you can hide the panel in the IH-Lab gallery you are working on: click the border between the panel and the preview window.

5.2 Display Options of an IH-Lab Gallery

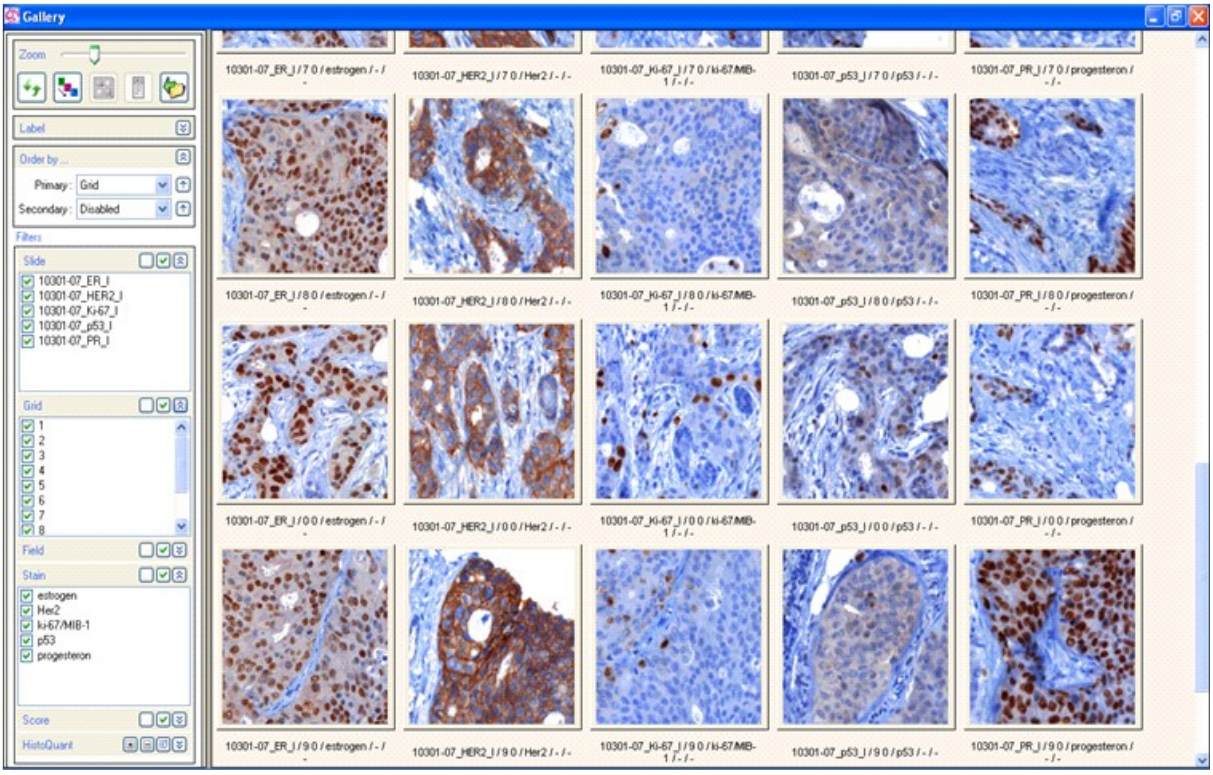
In the gallery we can determine the size of the preview of the fields by setting the **Zoom**. The gallery provides multiple visualization options for the IH-Lab project, which we can change by many ways at the left side of the window.

By default, IH-Lab shows the annotations (the grid fields) as thumbnails in the following order of importance: 1. group by slides 2. group by grids 3. group by fields

To modify the display order, set the primary and secondary ordering principle in the **Order by...** area and click on to refresh the gallery.

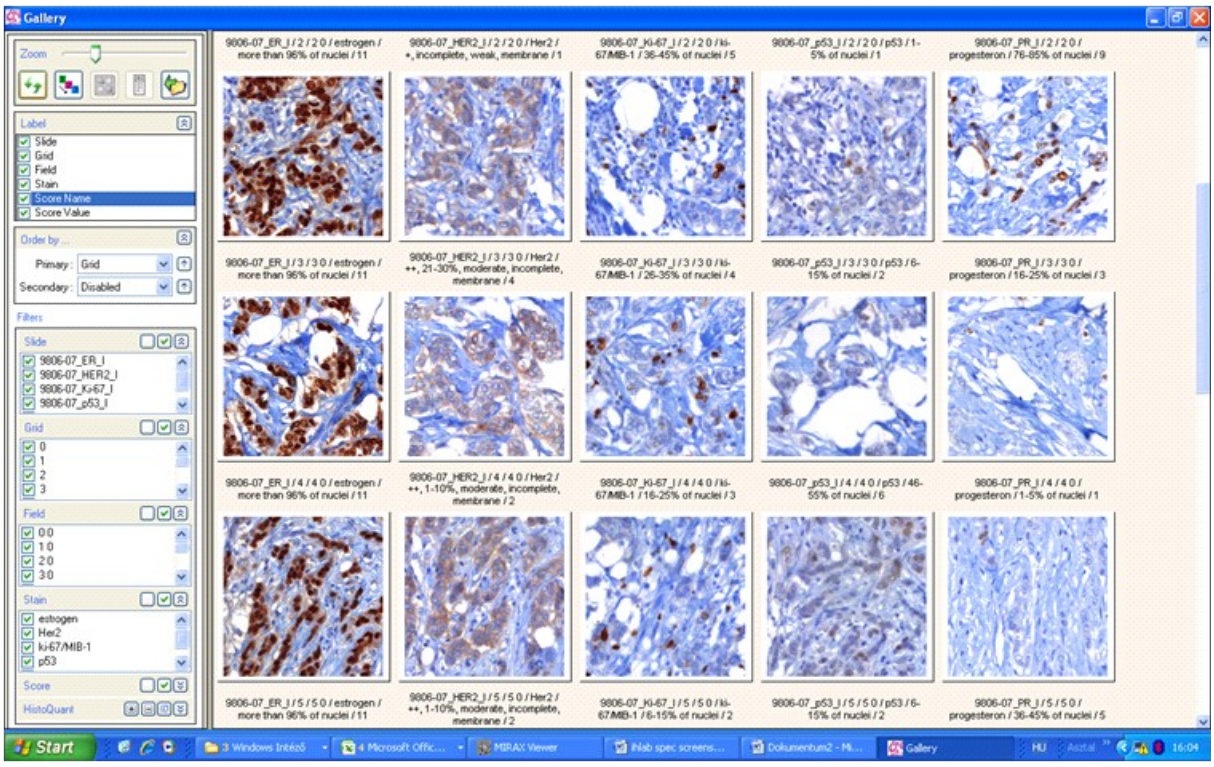
If you are evaluating an IH-Lab project from the same specimen, in which the slides were handled with different stains, order the thumbnails by **Stain**.

If you want to check your consistency in scoring, order the thumbnails by **Score**.



5.3 Setting Which Labels Are Visible in the HTML Export

You can change the content of the captions visible above the fields in the IH-Lab Gallery. For example, you might want to show the slide number and the grid field number in the caption of the thumbnails if you plan to export an HTML gallery from your IH-Lab project because this is the only way to show the scores in the output.


Gallery
Zoom
Label
Slide
Grid
Field
Stain
Score Name
Score Value
Order by ...
Primary: Grid
Secondary: Disabled
Filters
Slide
9806-07_ER_I
9806-07_HER2_I
9806-07_Ki-67_I
9806-07_p53_I
Grid
0
1
2
3
Field
0 0
1 0
2 0
3 0
Stain
estrogen
Her2
ki-67/MIB-1
p53
Score
HistoQuant
9806-07_ER_I / 2 / 2 0 / estrogen / more than 96% of nuclei / 11
9806-07_HER2_I / 2 / 2 0 / Her2 / +, incomplete, weak, membrane / 1
9806-07_Ki-67_I / 2 / 2 0 / ki-67/MIB-1 / 36-45% of nuclei / 5
9806-07_p53_I / 2 / 2 0 / p53 / 1-5% of nuclei / 1
9806-07_PR_I / 2 / 2 0 / progesteron / 76-85% of nuclei / 9
9806-07_ER_I / 3 / 3 0 / estrogen / more than 96% of nuclei / 11
9806-07_HER2_I / 3 / 3 0 / Her2 / ++, 21-30%, moderate, incomplete, membrane / 4
9806-07_Ki-67_I / 3 / 3 0 / ki-67/MIB-1 / 26-35% of nuclei / 4
9806-07_p53_I / 3 / 3 0 / p53 / 6-15% of nuclei / 2
9806-07_PR_I / 3 / 3 0 / progesteron / 16-25% of nuclei / 3
9806-07_ER_I / 4 / 4 0 / estrogen / more than 96% of nuclei / 11
9806-07_HER2_I / 4 / 4 0 / Her2 / ++, 1-10%, moderate, incomplete, membrane / 2
9806-07_Ki-67_I / 4 / 4 0 / ki-67/MIB-1 / 16-25% of nuclei / 3
9806-07_p53_I / 4 / 4 0 / p53 / 46-55% of nuclei / 6
9806-07_PR_I / 4 / 4 0 / progesteron / 1-5% of nuclei / 1
9806-07_ER_I / 5 / 5 0 / estrogen / more than 96% of nuclei / 11
9806-07_HER2_I / 5 / 5 0 / Her2 / ++, 1-10%, moderate, incomplete, membrane / 2
9806-07_Ki-67_I / 5 / 5 0 / ki-67/MIB-1 / 6-15% of nuclei / 2
9806-07_p53_I / 5 / 5 0 / p53 / 6-15% of nuclei / 2
9806-07_PR_I / 5 / 5 0 / progesteron / 36-45% of nuclei / 5
Start
3 Windows Intéző
4 Microsoft Offic...
MIRAX Viewer
iNlab spec screens...
Dokumentum2 - Mi...
Gallery
HU
16:04

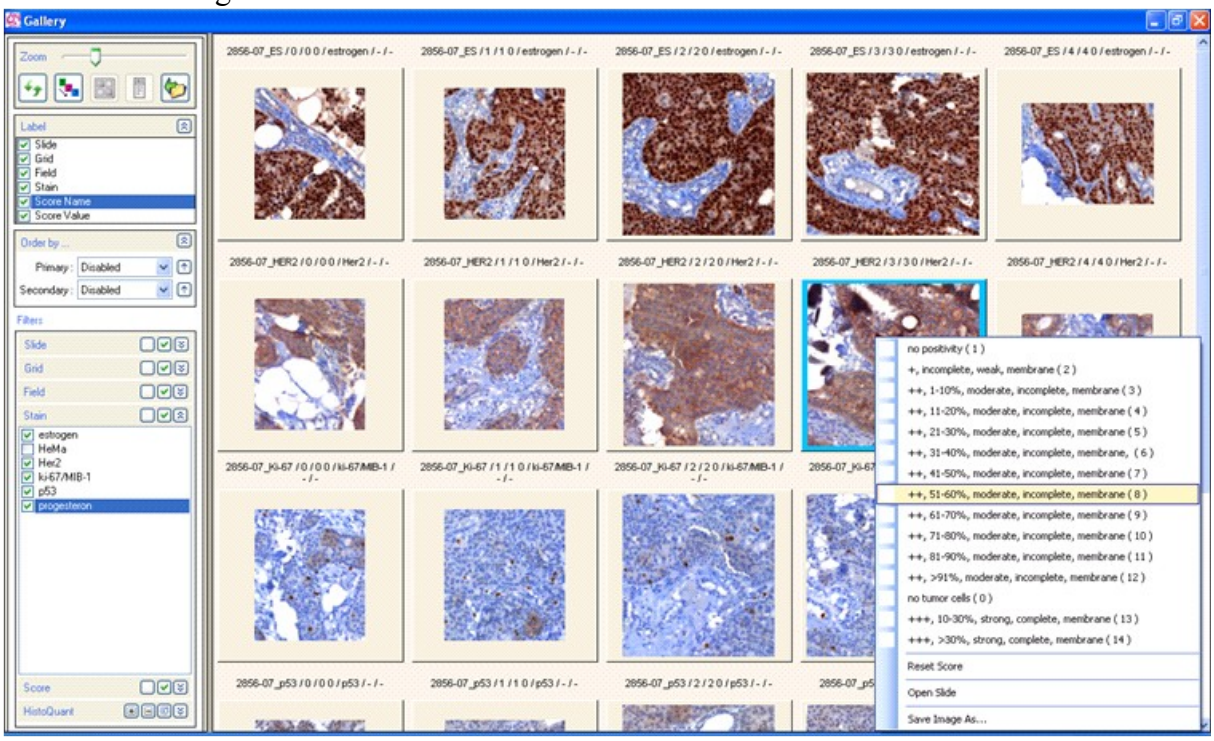
6 Scoring Slides in an IH-Lab Project

6.1 Scoring Options:

- From the gallery
- sequential scoring
- per slide

6.1.1 Scoring in the IH-Lab Gallery

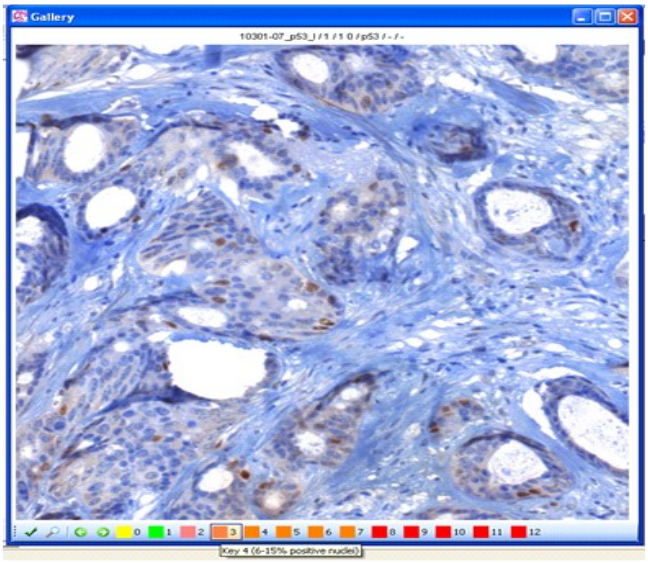
1. Open the IH-Lab project.
2. Click **Gallery** in the project menu and display the thumbnails.
3. Score the grid fields:



- To change the order of the display before you start scoring: change the grouping settings in the **Order by** frame.
- To score the annotations in the order they are displayed in the gallery start sequential scoring.
- To score the annotations one-by-one: right-click each thumbnail and select the score from the shortcut menu.

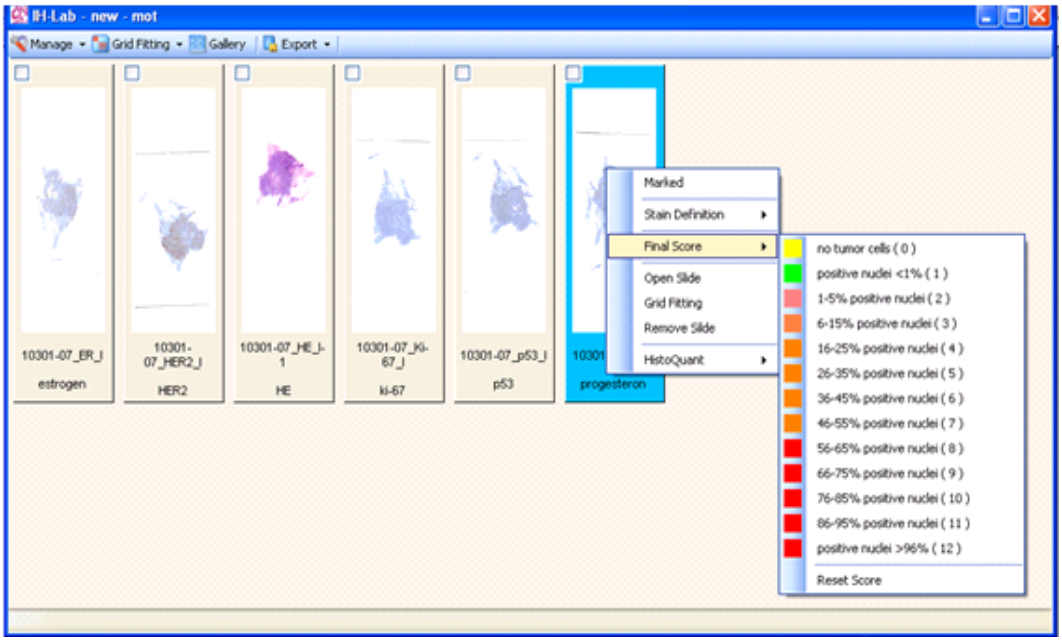
6.1.2 Sequential Scoring

Sequential scoring is a comfortable way to go through and score each fields of the grids. You can launch this option by clicking in the Gallery. In the window appears only one field for evaluation and by clicking the score at the bottom toolbar the next field is coming up automatically.



6.1.3 Scoring a Single Slide

1. Open the IH-Lab project.
2. Right-click the slide that you want to score, select **Final Score** from the shortcut menu, and then select the score.



6.2 Marking Slides

You can flag slides in IH-Lab projects, for example to mark the ones that you have already scored.

1. Start the IH-Lab project.

2. Right-click the slide that you want to flag and select **Marked** from the shortcut menu.

7 Exporting Information from an IH-Lab Project

7.1 Exporting Data from an IH-Lab Project to a CSV File

In the export menu you can export your added values and scores to a CSV (comma-separeted vaule) file, that you can open in a spreadsheet, or import into a database. You can export individual grid field values (data about this project) or you can export the data from this project into a long-term CSV file (a cumulative data file).

- Open the IH-Lab project.
- From the menu, select Export and then select what data you want to export from the IH-Lab project:

Full export: data from the IH-Lab project, including a summary line, exported into its own CSV file.

Cumulative export: summary line appended to an existing CSV file in which you compile longitudinal or otherwise cumulative data.

7.2 Exporting an HTML File

Tip!

IH-Lab does not export the scores to the HTML file. Add the scores to the annotation label before you export the IH-Lab Gallery and you will see the score above each annotation.

1. Open the IH-Lab gallery.
2. Click on the top panel and then select **Save to HTML** from the shortcut menu.

7.3 Exporting an image

Listed images can be exported at arbitrary magnification into a HTML file, or save all listed pictures as image files (BMP, JPEG or PNG).

1. Open the IH-Lab gallery.
2. Click on the panel and then select **Save to Picture(s)** from the shortcut menu.

3. Set the magnification level and the format and then save the pictures.

7.4 Exporting One Annotation as a Picture from an IH-Lab Gallery

Listed images can be exported at arbitrary magnification into a HTML file, or save all listed pictures as image files (BMP, JPEG or PNG).

1. Open the IH-Lab gallery.
2. Right-click the annotation that you want to export.
3. Set the magnification level and the format and then save the picture.

8 Working with IH-Lab in Multi-User Mode

If several users are assigned to an IH-Lab project, the application automatically creates a consolidator user, who can see the scores entered by all other users and can finalize the score into one consolidated report.

Consolidator's Scoring offers the following options to consolidators:

- The consolidator can see and score only those fields (grid cells) that received the same score from all users.
- The consolidator can decide whether to take the lower or the upper median if there are even-numbered users assigned to the project.

9 Using HistoQuant and IH-Lab Together

You can use IH-Lab and HistoQuant together to perform measurements regarding the markers used on the slides. If you first score a slide set with IH-Lab, you can create and run a HistoQuant algorithm on the grid annotations to cross-check the manual scores or to support the Consolidator's decisions in hard-to-decide cases. For example:

1. Evaluate the cuts from the specimen with possibly cancerous cells and score them manually as positive/negative in IH-Lab.

2. Create an algorithm on an annotation with easy-to-decide positive/negative stains in HistoQuant for the same positive/negative parameter and run the algorithm on all fields in the IH-Lab project.

3. Compare the results from the manual scoring and from the HistoQuant algorithm and adjust the manual scores or refine the HistoQuant algorithm as necessary.

If you first run the HistoQuant algorithm on the grid fields or annotations inside the fields, the HistoQuant measurements make it is easer for the Evaluators to score the IH-Lab Gallery.